

Centrioles with Ten Singlets in Spermatozoa of the Parasitic Nematode *Heligmosomoides polygyrus*

Aïcha MANSIR & Jean-Lou JUSTINE

Laboratoire de Biologie Parasitaire, Protistologie, Helminthologie,
Muséum National d'Histoire Naturelle, 61 rue Buffon, F-75231 Paris cedex 05, France

ABSTRACT

The spermatozoon of *Heligmosomoides polygyrus* is elongate and aflagellate. The nucleus, in a posterior position, is arrowhead-shaped and has an anterior fossa. Two centrioles, which are the only microtubular organelles of the mature spermatozoon, are mutually perpendicular in the nuclear fossa. These two centrioles are made up of 10 singlets, closely arranged, forming an ellipse 140 x 160 nm. This is the first description of a centriole with 10 singlets. Immunocytochemistry demonstrates that spermatids have a transitory system of microtubules converging toward the centrioles. Spermatozoal centrioles may provide additional character for the understanding of nematode phylogeny.

RÉSUMÉ

Centrioles à dix singulets dans les spermatozoïdes du nématode parasite *Heligmosomoides polygyrus*

Le spermatozoïde de *Heligmosomoides polygyrus* est allongé et aflagellé. Le noyau, placé postérieurement, est en forme de pointe de flèche et présente une fossette à sa partie antérieure. Deux centrioles, qui sont les seuls organites composés de microtubules dans le spermatozoïde mûr, sont disposés perpendiculairement dans la fossette nucléaire. Ces deux centrioles sont composés de 10 singulets juxtaposés, formant une ellipse de 140 x 160 nm. Ceci est la première description d'un centriole à 10 singulets. L'immunocytochimie montre que les spermatides possèdent un système transitoire de microtubules convergeant vers les centrioles. Les centrioles des spermatozoïdes peuvent fournir de nouveaux caractères pour la compréhension de la phylogénie des Nématodes.

The spermatozoa of the Nematoda are all aflagellate. Axonemes are always absent but microtubules are sometimes present during spermiogenesis. In mature spermatozoa, microtubules have been described only in a few species [6]. Table 1 is a list of species in which the spermatozoon has been described by electron microscopy.

In *Heligmosomoides polygyrus*, a parasitic nematode often used in laboratory experiments, we found an outstanding centriolar structure, which is described here. The spermatozoon has been described previously in this species [81, 84], but centriolar structure was not addressed.

In addition, the present paper gives some information on the fate of the microtubular system of spermatids, observed by mean of immunocytochemistry of tubulin.

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MATERIALS AND METHODS

Material. Samples of *Heligmosomoides polygyrus* (Dujardin, 1845) were obtained from laboratory white mice, strain CD1. This species is sometimes named *Nematospiroides dubius* but this is a junior synonym [20, 21]. The strain used here belongs to the subspecies *Heligmosomoides polygyrus bakeri* Durette-Desset, Kinsella & Forrester, 1972 [19], originally collected from *Peromyscus maniculatus* in North America and transferred to laboratory mice, strain CD1, in which it has been maintained for more than twenty years [46]. Adults were collected two to four weeks after oral infestation with 300 larvae. Males were isolated under a binocular microscope and kept for a few hours in salt water (NaCl 9%).

Electron microscopy. Living specimens were placed in cold (4 °C) fixative, the head being immediately cut to allow the fixative to penetrate into the body. Specimens were fixed for 1 h in 2% glutaraldehyde in a buffer solution of 0.1 M sodium cacodylate at pH 7.2 at 4°C. After rinsing in the same buffer, the worms were postfixed for 1 h in 1% osmium tetroxide in the same buffer, dehydrated in ethanol and propylene oxide, and embedded in SPURR's medium [69]. Ultrathin sections were contrasted with DADDOW's method [15], and observed with a Hitachi H600 electron microscope.

Immunocytochemistry. Germ cells were obtained by dissecting each male in a drop of PBS (phosphate buffer saline, Sigma) on a pit slide previously washed with alcohol and acetone. The genital system was gently pressed with thin needles to release germ cells. Slides were kept in a humid chamber for 1-2 hours to allow cells to sink and adhere to the slide. The PBS was then removed and replaced by a drop of 3.7% formaldehyde in PBS for fixation. After 15 min the pit was rinsed with PBS (3 x 5 min). Cells were then permeabilized in 0.1% Triton X-100 in PBS and rinsed (PBS, 3 x 5 min). Non-specific antigens were blocked with 2% Bovine Serum Albumin (Sigma) in PBS (BSA-PBS) for 45-90 min at room temperature. Without intermediary washing, a monoclonal anti-tubulin antibody (anti-alpha-tubulin, clone DM 1A, Sigma, or anti-beta-tubulin, clone TUB 2.1, Sigma or anti-acetylated-tubulin, clone 6-11B-1, Sigma) diluted at 1/200 in BSA-PBS was applied for 40 min at room temperature. After rinsing (PBS 3 x 5 min) the FITC-conjugated antibody (Goat anti-mouse, Nordic, 1/40 in PBS) was applied for 40 min at room temperature. After a final wash (PBS 3 x 5 min), mounting was done in Citifluor (Citifluor Ltd, London, UK) and slides were sealed with nail enamel. Controls were done by omitting the first antibody or by using a non-relevant mouse antibody. Observations were made with a Nikon Optiphot epifluorescence microscope equipped with mercury lamp and a single band Nikon filter for FITC channel (B-2A).

RESULTS

Electron microscopy of mature spermatozoa (Fig. 1)

The spermatozoon of *Heligmosomoides polygyrus*, observed in the testis, is an elongate and aflagellate cell (Fig. 1a), about 17 µm in length and 3 µm in width. It comprises three regions: 1. The anterior region, opposite the nucleus, is devoid of organelles and contains a fibrillar cytoplasm. It is known that this region produces pseudopods in activated spermatozoa and is functionally anterior [81, 84]. 2. The median region contains round mitochondria and membranous organelles, which are similar to those described in other nematode spermatozoa. 3. The posterior region contains an arrowhead-shaped nucleus, with highly condensed chromatin. The nuclear envelope is absent, as in most nematodes. The anterior fossa of the nucleus contains two centrioles, which are the only microtubular elements of the mature spermatozoon.

The two centrioles have different orientations (Fig. 1b, c). One is aligned or slightly oblique along the longitudinal axis of the spermatozoon, and will be here termed longitudinal. The other centriole is perpendicular to the other and will be termed perpendicular.

The perpendicular centriole is made up of 10 singlets (Fig. 1b-e). Each singlet is in contact with its two neighbours. The singlets are not arranged in a perfect circle, but in an ellipse (140 x 160 nm). Ten dense peripheral elements are visible at the periphery of the centriole: relatively

FIG. 1. — Electron microscopy of *Heligmosomoides polygyrus* spermatozoon, showing centrioles with 10 singlets.
a: Longitudinal section of spermatozoon, showing anterior region (A), median region (M) and posterior region with nucleus (N). **b, c:** Sections showing the two centrioles in the nuclear fossa. **b:** Longitudinal, relatively thick, section of spermatozoon. The perpendicular centriole (right) shows 10 peripheral spokes (arrows); **c:** Transverse section. **d, e:** Perpendicular centriole in longitudinal section of the spermatozoon. Note the 10 peripheral dots. **f-h:** Longitudinal centriole. **f:** Longitudinal section of nucleus, showing centriole in nuclear fossa. **g, h:** Transverse section of spermatozoon, showing longitudinal centriole with 10 singlets and peripheral dots. In e and h, note elliptic shape of centriole and singlets closely associated. a, x 12 000; b, d, x 45 000; c, x 30 000; e, x 100 000; f, x 15 000; g, x 34 000; h, x 150 000.

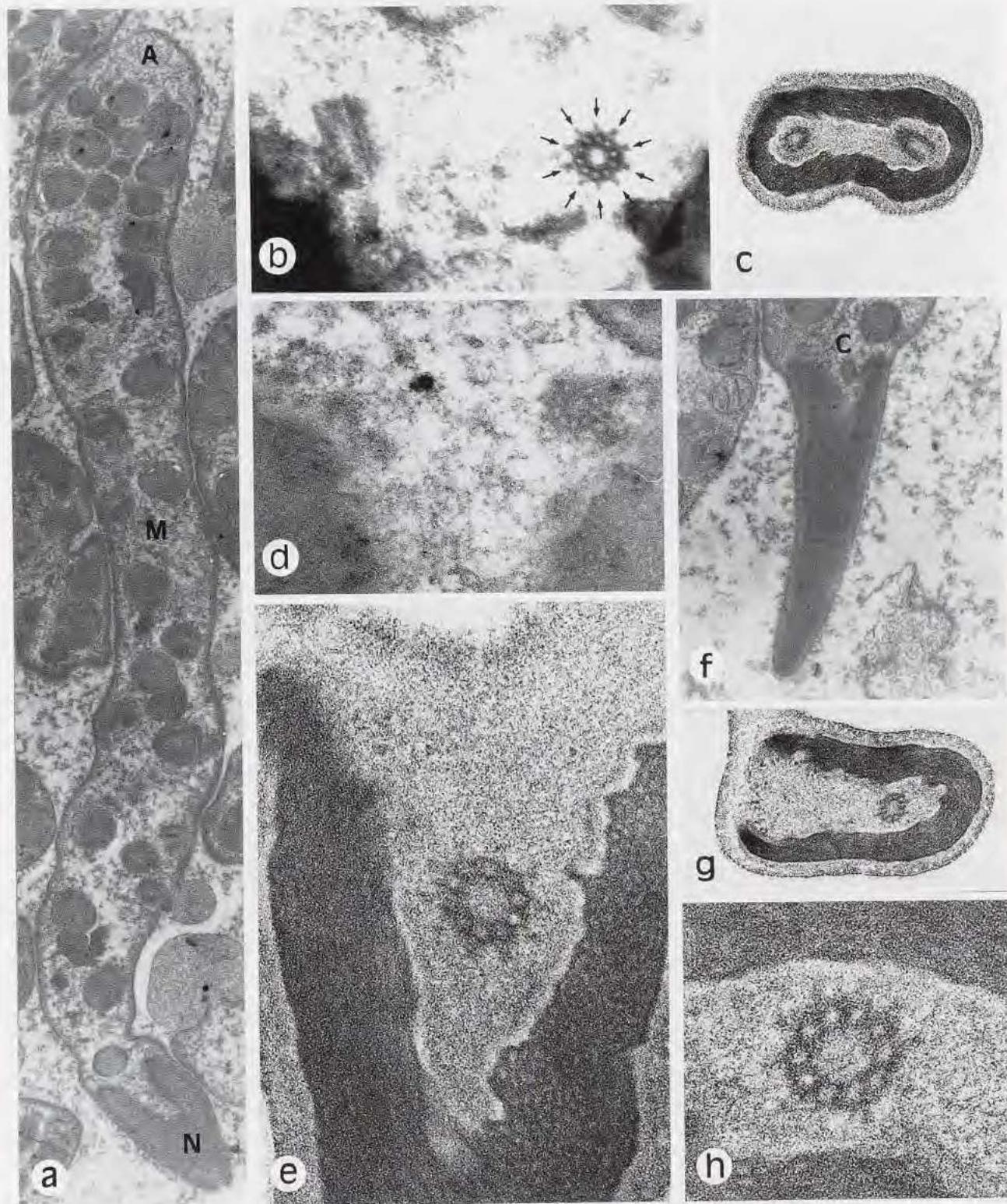


TABLE 1. — Spermatozoa of nematodes described by electron microscopy. Classification according to INGLIS (1983) [32]. Many orders of the Nematoda have not been studied for spermatozoal ultrastructure and thus are not cited here.

Class Rhabditia		Subclass Tylenchia	
Subclass Rhabditia		Order Tylenchida	
Order Rhabditida		<i>Aphelenchoides blastophthorus</i>	[65]
<i>Caenorhabditis elegans</i>	[48, 51, 57-59, 64, 77, 78, 80]	<i>Ekphymatodera thomasoni</i>	[11]
<i>Cephalobus cf quinilineatus</i>	[6]	<i>Heterodera (Glob.) rostochiensis</i>	[67]
<i>Heterorhabditis bacteriophora</i>	[28, 52]	<i>Heterodera (Globodera) virginiae</i>	[67]
<i>Neoaplectana intermedia</i>	[29]	<i>Heterodera (Heterodera) avenae</i>	[67]
<i>Rhabditis pellio</i>	[7]	<i>Heterodera (Heterodera) schachtii</i>	[67]
Order Oxyurida		<i>Meloidodera floridensis</i>	[10]
<i>Aspiculuris tetraptera</i>	[41]	<i>Meloidogyne acronea</i>	[66]
Order Strongylida		<i>Meloidogyne arenaria</i>	[66]
<i>Ancylostoma caninum</i>	[24, 71-73]	<i>Meloidogyne graminicola</i>	[66]
<i>Angiostrongylus cantonensis</i>	[24]	<i>Meloidogyne hapla</i>	[27, 66]
<i>Angiostrongylus cantonensis</i>	[24]	<i>Meloidogyne incognita</i>	[66]
<i>Heligmosomoides polygyrus</i>	[81, 84] This study	<i>Meloidogyne oryzae</i>	[66]
<i>Metastrongylus apri</i>	[14]	<i>Tylenchulus semipenetrans</i>	[6]
<i>Nematodirus battus</i>	[44]	<i>Verutus volvingensis</i>	[10]
<i>Nippostrongylus brasiliensis</i>	[33, 82, 83]		
<i>Protostrongylus rufescens</i>	[4]		
Subclass Diplogasteria		Class Enoplea	
Order Diplogasterina		Subclass Enoplia	
<i>Panagrellus silusiae</i>	[50]	Order Enoplida	
Order Ascaridida		<i>Deontostoma californicum</i>	[85]
<i>Ascaris megalcephala</i>	[22]	<i>Enoplus anisospiculus</i>	[87]
<i>Ascaris suum</i>	[1-3, 9, 12, 13, 23, 24, 26, 38, 56, 63]	<i>Enoplus demani</i>	[87]
<i>Heterakis gallinarum</i>	[40]	<i>Mesacanthion hirsutum</i>	[6]
<i>Polydelphis</i> sp.	[24]	Subclass Dorylaimia	
<i>Pseudoterranova decipiens</i>	[42]	Order Dorylaimida	
<i>Rhigonema madecassum</i>	[76]	<i>Xiphinema diversicaudatum</i>	[6]
Order Spirurida		<i>Xiphinema theresiae</i>	[39, 74]
<i>Brugia pahangi</i>	[9]	Order Dioctophymatida	
<i>Dipetalonema dessetae</i>	[43]	<i>Dioctophyma renale</i>	[24]
<i>Dipetalonema setariosum</i>	[45]	Order Mermithida	
<i>Dipetalonema viteae</i>	[25, 45]	<i>Gastromermis</i> sp.	[53]
<i>Dirofilaria immitis</i>	[24, 45]	Order Mononchida	
<i>Dirofilaria immitis</i>	[24]	<i>Mylonchulus nainitalensis</i>	[6]
<i>Gnathostoma</i> sp.	[24]	Order Trichurida	
<i>Litosomoides carinii</i>	[45]	<i>Capillaria hepatica</i>	[47]
<i>Loa loa</i>	[79]	<i>Trichinella nativa</i>	[30, 31]
<i>Physaloptera</i> sp.	[24]	<i>Trichinella pseudospiralis</i>	[31]
		<i>Trichinella spiralis</i>	[30, 31, 68, 70]
		<i>Trichuris muris</i>	[34, 35]
Class Chromadorea		Class Chromadorea	
Subclass Monohysteria		Subclass Monohysteria	
Order Monhysterida		Order Monhysterida	
<i>Sphaerolaimus hirsutus</i>		<i>Sphaerolaimus hirsutus</i>	[37, 49]

thick sections (Fig. 1b) show a triangular spoke 30 nm long extending from the peripheral limit of two singlets, and thin sections (Fig. 1d, e) show a dot located at a distance of 30 nm, and located at the level of this limit. The centre of the centriole shows no dense structure.

The longitudinal centriole (Fig. 1b, c, f-h) is also made up of 10 singlets and has the 10 peripheral elements. Lengths ranging from 200 nm (Fig. 1f) to 330 nm (Fig. 1b) have been found, possibly reflecting a change of length during the maturation of the spermatozoon.

Immunocytochemistry of tubulin in spermatids and spermatozoa (Fig. 2)

The immunolocalization of tubulin in mature spermatozoa gives absolutely no reaction, and thus is not illustrated here. However, spermatids show the presence of tubulin. Early elongate spermatids (Fig. 2a) show a heavy labelling of tubulin around the nucleus and in the elongating part of the cytoplasm. The most intense labelling is found in a triangular region located inside the anterior fossa of the nucleus. More advanced spermatids (Fig. 2b) show labelling only in the fossa and in a longitudinal central core in the cytoplasm. Finally, only a few parallel longitudinal microtubules are visible, labelling of which is most intense in the fossa (Fig. 2c). Mature spermatids (Fig. 2d) show no labelling.

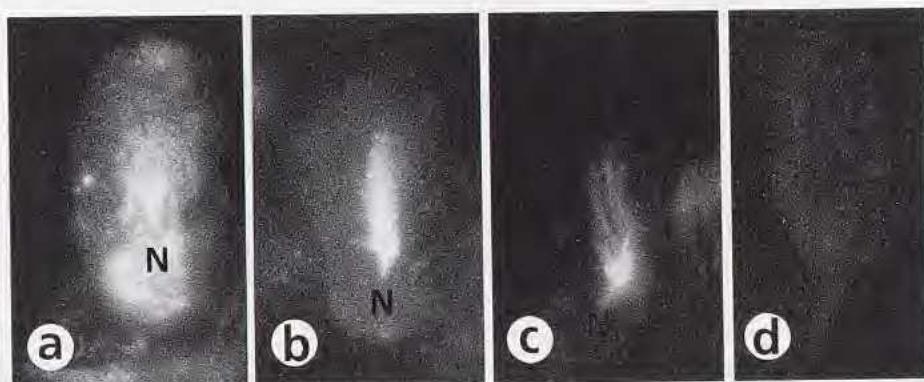


FIG. 2. — Immunocytochemistry of alpha-tubulin in spermatids of *Heligmosomoides polygyrus*. a-d: Successive stages of spermiogenesis, showing the decreased amount of tubulin in spermatids. The nuclear fossa is strongly labelled, thus indicating that the centriolar region located here may act as a microtubule organizing centre. Mature spermatozoa are not labelled and thus are not pictured here. a-d, $\times 2\,500$.

This labelling is consistent with the view that the centriolar region, located in the nuclear fossa, plays the role of a microtubule organizing centre (MTOC). However, centrioles in mature spermatids and spermatozoa are no longer labelled by the antibodies. This could be due to a penetration problem, since the centrioles are deeply located in the fossa in the mature spermatozoa and are embedded in an electron-dense material. An alteration of tubulin antigenicity in mature spermatozoa cannot, however, be excluded.

The labelling illustrated in Fig. 2 was performed with an anti-alpha tubulin antibody. The anti beta-tubulin antibody gives a similar pattern. The anti-acetylated tubulin antibody gave no labelling at any stage.

DISCUSSION

The spermatozoon of *Heligmosomoides polygyrus* has been previously described by WRIGHT & SOMMERVILLE [81, 84]. Our study confirms their observation on gross morphology and on the presence of microtubules in spermatids, which are no longer present in mature spermatozoa. However, the centrioles were not described in their study.

Centrioles have been described in nematode spermatozoa in a limited number of species (Table 2).

TABLE 2. — Centrioles in nematode spermatozoa.

Species	Centriole structure	Reference
<i>Heterakis gallinorum</i>	9 singlets	[40]
<i>Dipetalonema viteae</i>	9 singlets	[45]
<i>Caenorhabditis elegans</i>	9 singlets	[80]
<i>Trichinella spiralis</i>	9 singlets	[68]
<i>Sphaerolaimus hirsutus</i>	9 singlets	unpublished
<i>Ascaris megalcephala</i>	9 singlets	[22]
<i>Nippostrongylus brasiliensis</i>	9 singlets	[33, 83]
<i>Gastromermis</i> sp. (spermatid)	9 doublets	[53]
<i>Heligmosomoides polygyrus</i>	10 singlets	Present study

Table 2 demonstrates that singlets are the usual structure found in nematode spermatozoal centrioles. The description of doublets in *Gastromermis* refers only to spermatids [53] and the structure is not described in mature spermatozoa. It is not unlikely that the doublets are simplified into singlets in mature spermatozoa in this species; such a process is known in many Platyhelminthes (see [36]).

The number of singlets in nematode sperm centrioles is generally 9. The centriole of *Nippostrongylus* has been described as having 18 singlets by JAMUAR [33], but examination of the photographs suggests that it is made of 9 singlets separated by regular spaces, as later correctly described by WRIGHT & SOMMERVILLE [83]. The case of *Heligmosomoides polygyrus* described here is the first case with 10 singlets, and therefore 10-fold symmetry.

The 9-fold symmetry is almost ubiquitous in axonemes. A few exceptions have been noted: axonemes made up of 3 [54], 6 [62], 8 [55], 12 [5, 75], 13 [17, 86], 14 [5, 18], 16 doublets [18] have been described in spermatozoa of certain animals. Axonemes with a 9+“1” structure (nine doublets) but with centrioles made up of 18 singlets have been recently described in a flatworm [36]. The only known case of a ten-fold symmetry in a spermatozoal axoneme has been very recently described by DALLAI *et al.* [16] in the spermatozoa of a dipteran, which has a 10+0 structure. DALLAI *et al.* (1995) [16] have remarked that “in order to accommodate a tenth doublet, the axoneme either must increase its cross-sectional diameter or the doublets have to be more closely packed. The former alternative seems to be realized as the axoneme is seen to have an elliptic shape...”. In *Heligmosomoides polygyrus* centrioles, both conditions (closer microtubules and elliptic shape) are found.

It is known that the ciliary apparatus is generally very reduced in nematodes [8]: flagella are absent from spermatozoa, motile cilia are unknown, and an axonemal structure is found only in sensory cells. Moreover, sensory cilia of nematodes often show a deviation from the 9+2 pattern. Cilia with 10+0 structure (10 peripheral doublets) have been described in larval *Haemonchus contortus* [60].

In spermatozoa which have a flagellum, the presence of a centriole is correlated with the existence of the flagellum. In the Nematoda, flagella are never present and the role of the centrioles may be questioned. Centrioles seem to act as MTOC during spermiogenesis, but might, on first consideration, appear redundant in mature sperm. Their presence in mature sperm may indicate a role during fertilization. However, the participation of the paternal centriole during fertilization is not ascertained in all animal species [61]. In the nematodes, a comparative study of the role of centrioles in fertilization in species with centrioles with 9 singlets and in *Heligmosomoides polygyrus* with 10 singlets would be interesting.

Spermatozoa of nematodes have been tentatively used for the understanding of phylogeny by BACCETTI *et al.* [6]. The position of the centrioles may provide additional characters. For instance, if we consider only spermatozoa with elongate shape, the position of centrioles is different in *Gastromermis*, where they are at the distal (posterior) tip of the nucleus [53], whereas they are at the anterior extremity of the nucleus in the Trichostrongyloidea [33, 44, 82-84]. The symmetry of the centrioles may provide further useful character.

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